

CLAIMS

✓ 1. A method of synthesizing nucleic acid having complementary nucleotide sequences linked alternately in a single-stranded chain, comprising:

a) the step of giving nucleic acid which is provided at the 3'-terminal thereof with a region F1 capable of annealing to a part F1c in the same chain and which upon annealing of the region F1 to F1c, is capable of forming a loop containing a region F2c capable of base pairing,

b) the step of performing synthesis of a complementary chain wherein the 3'-terminal of F1 having annealed to F1c serves as the origin of synthesis,

c) the step of annealing, to a region F2c, of an oligonucleotide provided with the 3'-terminal thereof with F2 consisting of a sequence complementary to the region F2c, followed by synthesis, with said oligonucleotide as the origin of synthesis, of a complementary chain by a polymerase catalyzing the strand displacement reaction of synthesizing a complementary chain to displace the complementary chain synthesized in step b), and

d) the step of annealing, to the complementary chain displaced in step c) to be ready for base pairing, of a polynucleotide provided at the 3'-terminal thereof with a sequence complementary to an arbitrary region in said chain synthesized in step c), followed by synthesis, with said 3'-terminal as the origin of synthesis, of a complementary chain by a polymerase

catalyzing the strand displacement reaction of synthesizing a complementary chain to displace the complementary chain synthesized in step c).

2. The method according to ~~claim 1~~, wherein in step d), the origin of synthesis is a region R1 present at the 3'-terminal in the same chain and capable of annealing to a region R1c, and a loop containing the region R2c capable of base pairing is formed by annealing R1 to R1c.

3. An oligonucleotide composed of at least two regions X2 and X1c below, and X1c is linked to the 5'-side of X2,

X2: a region having a nucleotide sequence complementary to an arbitrary region X2c in nucleic acid having a specific nucleotide sequence, and

X1c: a region having substantially the same nucleotide sequence as in a region X1c located at the 5'-side of the region X2c in nucleic acid having a specific nucleotide sequence.

4. The method according to claim 1, wherein the nucleic acid in step a) is second nucleic acid provided by the following steps:

i) the step of annealing, to a region F2c in nucleic acid serving as a template, of a region F2 in the oligonucleotide described in claim 3 wherein the region X2 is a region F2 and the region X1c is a region F1c,

ii) the step of synthesizing first nucleic acid having a nucleotide sequence complementary to the template wherein F2

in the oligonucleotide serves as the origin of synthesis,
iii) the step of rendering an arbitrary region in the first nucleic acid synthesized in step ii) ready for base pairing, and
iv) the step of annealing an oligonucleotide having a nucleotide sequence complementary to the region made ready for base pairing in the first nucleic acid in step iii), followed by synthesizing second nucleic acid with said oligonucleotide as the origin of synthesis and rendering F1 at the 3'-terminal thereof ready for base pairing.

5. The method according to claim 4, wherein the region enabling base pairing in step iii) is R2c, and the oligonucleotide in step iv) is the oligonucleotide described in claim 3 wherein the region X2c is a region R2c and the region X1c is a region R1c.

6. The method according to claim 4 or 5, wherein the step of rendering base pairing ready in steps iii) and iv) is conducted by the strand displacement synthesis of complementary chain by a polymerase catalyzing the strand displacement reaction of synthesizing complementary chain wherein an outer primer annealing to the 3'-side of F2c in the template and an outer primer annealing to the 3'-side of the region used as the origin of synthesis in step iv) for the first nucleic acid serve as the origin of synthesis.

7. The method according to claim 6, wherein the melting

temperature of each oligonucleotide and its complementary region in the template used in the reaction is in the following relationship under the same stringency:

(outer primer/region at the 3'-side in the template) \leq (F2c/F2 and R2c/R2) \leq (F1c/F1 and R1c/R1).

8. The method according to any one of claims 4 to 7, wherein the nucleic acid serving as the template is RNA, and the synthesis of complementary chain in step ii) is conducted by an enzyme having a reverse transcriptase activity.

9. A method of amplifying nucleic acid having complementary nucleotide sequences linked alternately in a single-stranded chain by repeatedly conducting the following steps:

A) the step of providing a template which is provided at the 3'- and 5'-terminals thereof with a region consisting of a nucleotide sequence complementary to each terminal region in the same chain and which upon annealing of these mutually complementary nucleotide sequences, forms a loop capable of base pairing therebetween,

B) the step of performing the synthesis of complementary chain wherein the 3'-terminal of said template annealed to the same chain serves as the origin of synthesis,

C) the step of annealing, to the loop portion, of an oligonucleotide provided at the 3'-terminal thereof with a complementary nucleotide sequence to a loop which among said loops, is located at the 3'-terminal site, followed by synthesis,

with the oligonucleotide as the origin of synthesis, of a complementary chain by a polymerase catalyzing the strand displacement reaction of synthesizing a complementary chain to displace the complementary chain synthesized in step B) to make the 3'-terminal thereof ready for base pairing, and

D) the step wherein the chain with the 3'-terminal made ready for base pairing in step C) serves as a new template.

10. The method according to claim 9, wherein the oligonucleotide in step C) is provided at the 5'-terminal thereof with a nucleotide sequence complementary to the 3'-terminal serving as the origin of synthesis in step B).

11. The method according to claim 10, further comprising the step where a complementary chain synthesized with the oligonucleotide in step C) as the origin of synthesis is used as a template in step A).

12. The method according to claim 9 wherein the template in step A) is synthesized by the method described in claim 5.

13. The method according to claim 1 or 9, wherein the strand displacement reaction of synthesizing complementary chain is carried out in the presence of a melting temperature regulator.

14. The method according to claim 13, wherein the melting temperature regulator is betaine.

15. The method according to claim 14, wherein 0.2 to 3.0 M betaine is allowed to be present in the reaction solution.

16. A method of detecting a target nucleotide sequence in a

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sample, which comprises performing an amplification method described in any one of claims 9 to 15 and observing whether an amplification reaction product is generated or not.

17. The method according to claim 16, wherein a probe containing a nucleotide sequence complementary to the loop is added to the amplification reaction product and hybridization therebetween is observed.

18. The method according to claim 17, wherein the probe is labeled on particles and aggregation reaction occurring upon hybridization is observed.

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19. The method according to claim 16, wherein an amplification method described in any one of claims 9 to 15 is conducted in the presence of a detector for nucleic acid, and whether an amplification reaction product is generated or not is observed on the basis of a change in the signal of the detector.

20. A method of detecting a mutation in a target nucleotide sequence by the detection method described in claim 16, wherein a mutation in a nucleotide sequence as the subject of amplification prevents synthesis of any one of complementary chains constituting the amplification method.

21. A kit for synthesis of nucleic acid having complementary chains alternately linked in a single-stranded chain, comprising the following elements:

i) the oligonucleotide described in claim 3 wherein the region F2c in nucleic acid as a template is X2c, and F1c located at

the 5'-side of F2c is X1c;

ii) an oligonucleotide containing a nucleotide sequence complementary to an arbitrary region in a complementary chain synthesized with the oligonucleotide in (i) as a primer;

iii) an oligonucleotide having a nucleotide sequence complementary to a region F3c located at the 3'-side of the region F2c in the nucleic acid serving as a template;

iv) a DNA polymerase catalyzing the strand displacement-type reaction of synthesizing complementary chain; and

v) a nucleotide serving as a substrate for the element iv).

22. The kit according to claim 21, wherein the oligonucleotide in ii) is the oligonucleotide described in claim 3 wherein an arbitrary region R2c in a complementary chain synthesized with the oligonucleotide in i) as the origin of synthesis is X2c, and R1c located at the 5' of R2c is X1c.

23. The kit according to claim 22, further comprising:

vi) an oligonucleotide having a nucleotide sequence complementary to a region R3c located at the 3'-side of the arbitrary R2c in a complementary chain synthesized with the oligonucleotide in i) as the origin of synthesis.

24. A kit for detection of a target nucleotide sequence, comprising a detector for detection of a product of nucleic acid synthetic reaction additionally in a kit described in any one of claims 21 to 23.

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